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Advanced 3rd Edition Immune Regulation Reagents

THESOURCE

Multimeric CD40L - A Potent B Cell Expansion Tool

MultimericCD40L™ is a high activity construct that very effectively simulates the natural membrane-assisted aggregation of CD40L. It provides a simple and equally potent alternative to CD40L + enhancer combinations. The CD40 Ligand Multimer mimics a T cell-dependent activation of B cells. AdipoGen Life Sciences provides unique CD40L (multimeric) proteins (mouse and human) that can be used for the activation and expansion of mouse and human B cells.

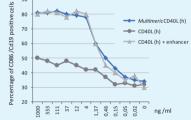
CD40L (human) (multimeric) (rec.)

AG-40B-0010 10 μg | 3 x 10 μg AG-40B-0010B NEW Biotin 10 μg | 3 x 10 μg

CD40L (mouse) (multimeric) (rec.)

AG-40B-0020 10 μg | 3 x 10 μg

FIGURE: CD40L (human) (multimeric) (rec.) (Prod. No. AG-40B-0010) does not need an enhancer to induce B cell activation



BULK

Potent Agonistic CD40 Antibody

anti-CD40 (mouse), mAb (FGK45) (preservative free)

Activates B and NK cells in vivo and in vitro!

AG-20B-0036PF $100 \mu g \mid 500 \mu g \mid 5 mg$

NEW

Fibrinogen-like Protein 1 (FGL1) - A Major Ligand of LAG-3

LAG-3 is a co-inhibitory immune checkpoint receptor that inhibits T cell response by binding to MHC-II. A recent study identifies Fibrinogen-like Protein 1 (FGL1), a molecule secreted by the liver and pancreas, as a major ligand for LAG-3 in both human and mouse. FGL1 binding to LAG-3 inhibits T cell response. FGL1 is upregulated in human cancers and high plasma FGL1 levels are associated with poor clinical outcome in patients treated with anti-PD1 therapy.

LIT: Fibrinogen-like Protein 1 Is a Major Immune Inhibitory Ligand of LAG-3: J. Wang, et al.; Cell 176, 334 (2019)

| PROTEINS | PID | SIZE | SOURCE | ENDOTOXIN | SPECIES |
|------------------------------------|-------------|----------------------------|---------------|------------|---------|
| NEW Fc (human):FGL1 (human) (rec.) | AG-40B-0184 | 10 μg 3 x 10 μg 100 μg | HEK 293 cells | <0.01EU/μg | Hu |
| NEW Fc (human):FGL1 (mouse) (rec.) | AG-40B-0185 | 10 μg 3 x 10 μg 100 μg | HEK 293 cells | <0.01EU/μg | Ms |
| NEW FGL1 (human) (rec.) (His) | AG-40B-0186 | 10 μg 3 x 10 μg | HEK 293 cells | <0.01EU/μg | Hu |



VISTA:COMP - Immunosuppressive In Vivo Agonist

VISTA is a new negative checkpoint regulator that potently suppresses T cell activation. Recently, it has been reported that recombinant VISTA protein needs to be **pentamerized to be active** as soluble ligand. The protein VISTA (mouse):COMP (mouse) (with the extracellular domain of mouse VISTA fused to the pentamerization domain from the cartilage oligomeric matrix protein (COMP), but not VISTA-Fc, functions as an immunosuppressive agonist *in vivo* inhibiting the proliferation of CD4⁺ T cells.

LIT: VISTA.COMP - an engineered checkpoint receptor agonist that potently suppresses T cell-mediated immune responses: A. Prodeus, et al.; JCI Insight 2, e94308 (2017)

| PROTEINS | PID | SIZE | SOURCE | ENDOTOXIN | SPECIES |
|---|-------------|-------|---------------|------------|---------|
| VISTA (mouse):COMP (mouse) (rec.) (His) | AG-40B-0181 | 50 μg | HEK 293 cells | <0.01EU/µg | Ms |
| NEW VISTA (human):COMP (mouse) (rec.) (His) | AG-40B-0183 | 50 μg | HEK 293 cells | <0.01EU/µg | Hu |

Expansion of T Regulatory Cells (Tregs)

IL-33 has emerged as a multifunctional protein with roles in driving potent type 1 and type 2 immunity as well as facilitating profound Treg expansion *in vitro* and *in vivo*. IL-33-expanded Tregs express the IL-33 receptor ST2 and classical markers associated with Treg phenotype and suppressor function. IL-33 may potentially be used to promote/maintain organ transplant tolerance or suppress autoimmunity and has been associated with Treg-mediated wound healing. Recently, IL-33 has also been found as a critical cytokine for the expansion of brain Treg cells that potentiates neurological recovery.

LIT: Poor repair of skeletal muscle in aging mice reflects a defect in local, interleukin-33-dependent accumulation of regulatory T cells: W. Kuswanto, et al.; Immunity 44, 355 (2016) • Expansion of regulatory T cells in vitro and in vivo by IL-33: B.M. Matta & H.R. Turnquist; Methods Mol. Biol. 1371, 29 (2016) • Brain regulatory T cells suppress astrogliosis and potentiate neurological recovery: M. Ito, et al.; Nature 565, 246 (2019)



NEW Highly Active Human IL-33 Proteins

IL-33 (oxidation resistant) (human) (rec.)

LIT: Oxidation of the alarmin IL-33 regulates ST2-dependent inflammation: E.S. Cohen, et al.; Nat. Commun. 6, ID8327 (2015)



Potent Th2 Immune Response Blocking Reagents

IL-33 and its receptor ST2 are involved in the Th2 immune response in allergic inflammation and parasite infection. AdipoGen Life Sciences provides powerful and specific reagents to block the IL-33/ST2 axis and the Th2 response.

HpARI (Alarmin Release Inhibitor) (rec.) (His)

AG-40B-0178 50 μ g | 3 x 50 μ g Natural IL-33 (human/mouse) interfering reagent from a parasite.

IL-33 (mouse), mAb (rec.) (blocking) (Bondy-1-1)

AG-27B-0013 $100 \mu g$ AG-27B-0013PF Preservative Free $100 \mu g \mid 500 \mu g \mid 1 mg$



Proteasome Inhibitors - The Widest Panel

Bortezomib [PS-341] | Carfilzomib [PR-171] | Delanzomib [CEP-18770] | Epoxomicin | Ixazomib [MLN2238] | Ixazomib citrate [MLN9708] | clasto-Lactacystin β -lactone | Lactacystin | ONX 0914 | Oprozomib [ONX 0912] | PI-1840 | Salinosporamide A | VR23

Proteasome inhibition with potent natural or synthetic small molecules has implications in a number of human diseases such as cancer, inflammation and ischemic stroke.



Plasma Cell Survival & Proliferation Blocking Antibodies

anti-BAFF (mouse), mAb (blocking) (Sandy-2)

AG-20B-0063 100 μg AG-20B-0063PF Preservative Free 100 μg | 500 μg

Functional Application: Depletion of B cells *in vivo.***.** Inhibition of mouse BAFF binding to BAFF-R and TACI (BCMA not tested); blocks BAFF activity in mice.

LIT: The B cell-stimulatory cytokines BLyS and APRIL are elevated in human periodontitis and are required for B cell-dependent bone loss in experimental murine periodontitis: T. Abe, et al.; J. Immunol. 195, 1427 (2015)

anti-APRIL (mouse), mAb (rec.) (blocking) (Apry-1-1)

AG-27B-0001 100 μg AG-27B-0001PF Preservative Free 100 μg AG-27B-0001B Biotin 100 μg

Functional Application: Depletion of plasma cells. Inhibits binding of mouse APRIL to mouse BCMA and TACI.

LIT: Production of the plasma-cell survival factor APRIL peaks in myeloid precursor cells from human bone marrow: T. Matthes, et al.; Blood 118, 1838 (2011)

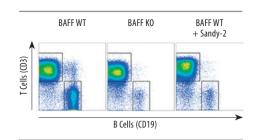


FIGURE: anti-BAFF (mouse), mAb (Sandy-2) (Prod. No. AG-20B-0063) blocks the action of endogenous BAFF *in vivo*.

METHOD: Wild type C57BL/6 mice were treated at day 0 (single administration) with monoclonal antibody anti-BAFF (mouse), mAb (Sandy-2) (at 2mg/kg). Lymph nodes were prepared at week 2 and analyzed by FACS for the presence of T (CD3) and B (CD19) cells. Untreated BAFF WT and KO mice were analyzed in parallel.

Multimeric & Mutant Cytokines with Increased Activity

IL-2 Superkine (Fc)

AG-40B-0111

10 μg | 3 x 10 μg

Compared to IL-2, IL-2 Superkine does not require CD25 to be active and induces superior expansion of cytotoxic CD8⁺ T and NK cells, leading to improved antitumor responses *in vivo*. IL-2 Superkine activates only poorly T regulatory cells (Tregs), meaning less toxic effects *in vivo*.

LIT: Exploiting a natural conformational switch to engineer an interleukin-2'superkine': AM. Levin, et al.: Nature **484**, 529 (2012)

FIGURE: Binding of IL-2 Superkine (Fc) (Prod. No. AG-40B-0111) to IL-2Rβ (human) is increased 10 fold compared to IL-2 (human):Fc (human).

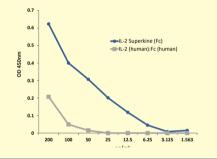
BAFF, Soluble (human) (60-mer) (rec.) (highly active)

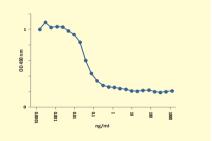
AG-40B-0112 10 μg | 3 x 10 μg

Biological Activity: Increases B cell survival/proliferation. Increases CD21/CD23 expression on B cells *in vivo*. **Activates BAFF-R, TACI and BCMA receptors.** Works at concentrations <20ng/ml.

LIT: Mutation of the BAFF furin cleavage site impairs B-cell homeostasis and antibody responses: C. Bossen, et al.; Eur. J. Immunol. 41, 787 (2011)

FIGURE: BAFF, Soluble (human) (60-mer) (Prod. No. AG-40B-0112) binds and activates BCMA receptor.





UNIQUE

Posttranslational Modification-Specific Tubulin Antibodies

| ANTIBODIES | PID | SIZE | ISOTYPE/SOURCE | APPLICATION |
|---|--------------|--------|----------------|-----------------|
| anti- $lpha$ -Tubulin (acetylated), mAb (TEU318) | AG-20B-0068 | 100 μg | Mouse IgG1 | ICC, WB |
| NEW anti-Tubulin (glycylated), pAb (Gly-pep1) | AG-25B-0034 | 100 μg | Rabbit | ICC, IP, WB |
| anti-Tubulin-GTP, mAb (rec.) (MB11) | AG-27B-0009 | 100 μg | Human IgG2κ | ICC |
| anti-Polyglutamylation Modification, mAb (GT335) | AG-20B-0020 | 100 μg | Mouse lgG1κ | EM, ICC, IP, WB |
| anti-Polyglutamylation Modification, mAb (GT335) (Biotin) | AG-20B-0020B | 100 μg | Mouse lgG1κ | ICC, IP, WB |
| anti-Polyglutamate chain (polyE), pAb (IN105) | AG-25B-0030 | 50 μg | Rabbit | ICC, WB |

Inflammasome Research Antibodies

STANDARDS

FROM THE EXPERTS & VALIDATED BY EXPERT LABORATORIES!

Unique NLRP3 Antibody

anti-NLRP3/NALP3, mAb (Cryo-2)

ICC, IHC, IP, WB

AG-20B-0014

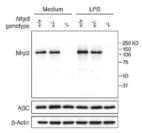


FIGURE: Mouse NLRP3 is detected in mouse macrophages using the monoclonal antibody to NLRP3 (Cryo-2) (Prod. No. AG-20B-0014).

PROTOCOLS FOR CASPER-1, CASPER-2, BALLY-1 AND CRYO-2:

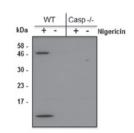
Measuring the inflammasome: O. Gross; Methods Mol. Biol. **844**, 199 (2012) • Measuring NLR Oligomerization I: Size Exclusion Chromatography, Co-immunoprecipitation, and Cross-Linking: S. Khare, et al.; Methods Mol. Biol. **1417**, 131 (2016)

Detect Activated p10 & p20 Caspase-1 by WB

anti-Caspase-1 (p10) (mouse), mAb (Casper-2) AG-20B-0044 anti-Caspase-1 (p20) (mouse), mAb (Casper-1) AG-20B-0042 anti-Caspase-1 (p20) (human), mAb (Bally-1) AG-20B-0048

FIGURE: Mouse caspase-1 (p10) is detected by immunoblotting using anti-Caspase-1 (p10) (mouse), mAb (Casper-2) (Prod. No AG-20B-0044).

METHOD: Caspase-1 was analyzed by Western blot in supernatants of differentiated bone marrow-derived dendritic cells (BMDCs) from wild-type and caspase-1-/- mice activated or not by 5μM nigericin (Prod. No. AG-CN2-0020) for 30 min. Supernatants (30μl) were separated by SDS-PAGE under reducing conditions, transferred to nitrocellulose and incubated with anti-Caspase-1 (p10) (mouse), mAb (Casper-2) (1μg/ml). Proteins were visualized by a chemiluminescence detection system.



Key Antibodies

| anti-Asc [Pycard], pAb (AL177) ICC, IHC, IP, WB, FUNC | AG-25B-0006 |
|---|-------------|
| anti-AIM2 (human), mAb (3B10) ICC, WB | AG-20B-0040 |
| anti-Caspase-4/11 (p20), mAb (Flamy-1) IP, WB | AG-20B-0060 |
| anti-RIG-I, mAb (Alme-1) IHC, IP, WB | AG-20B-0009 |
| anti-ZBP1, mAb (Zippy-1) ICC, IP, WB | AG-20B-0010 |

BULK

In vivo Inflammasome Inhibitor

MCC950 . sodium salt (>98%)

AG-CR1-3615

1 mg | 5 mg | 10 mg

From the Manufacturer of iNKT Stimulators

α-Galactosylceramide (>96%) [KRN 7000]

AG-CN2-0013 250 μg | 1 mg

UNIQUE

Highly Active DLL4 Notch Ligand



AG-40B-0176

10 μg | 100 μg

Interacts with human Notch1 with >20 fold increase in affinity relative to WT DLL4 (as confirmed by ELISA).

Visit www.adipogen.com for a Complete Panel of Notch-related Reagents!





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